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THE ACTION OF LEUCOCYTES TOWARD FOREIGN SUBSTANCES.[1]

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Among the many problems that yet await solution at the hands of the physiologist and histologist, those relating to the disappearance of so many leucocytes or white blood corpuscles from the animal body have long afforded a fruitful field for work. Under what conditions and by what means they are destroyed is as yet but partly known, and different theories are advanced as to the most probable method of this destruction. The constant relation, normally, that exists between the numbers of the white and red cells of the blood, in spite of the steady supply of white cells that is poured into the blood from the lymphatics, establishes the fact that somewhere there is as steady a drain on the numbers.

The nature of leucocytes as entities in the economy of the animal body is of especial importance in consideration of the second point and a careful study of these cells in a living condition helps one to realize their activities and powers. The ability of these cells to take up foreign substances by virtue of their amoeboid movement is very significant to the physiologist especially from a pathological standpoint. The great Russian morphologist, Metschnikoff, has based his phagocyte doctrine on the peculiarity given to these cells by the exercise of this power, giving to them in consequence an additional and important duty. They form, as it were, a guardian army in the animal body, ever alert and watchful for the invading enemy. A constant warfare is being waged between these leucocytes and all foreign material, organic or inorganic, that enters the system. By the process of ingestion the immediate influence

[1] This paper contains part of the results of an investigation carried on in the Histological Laboratory of Cornell University during last year. I wish to express my appreciation of the abundant material and facilities which were so generously put at my disposal. The whole paper afterwards received the first prize offered by the American Microscopical Society for original work in animal Histology.

of the substances is removed from the tissues. The balance of power continually wavers between these two hosts, on the one hand the leucocytes and on the other the different kinds of organisms and matter, injurious or non-injurious, to which the animal body is hourly exposed. If the invaders are too strong the results become evident in the sickness or perhaps death of the animal. But if the leucocytes are victorious, and are able to clear the system of the foreign substances, normal conditions are again established and with them the health of the individual. By no means is it necessary, however, that the conflict become apparent externally. This at least is the story picturesquely put as the founder of the "Phagocyte Theory" reads it. It may be rather extreme; certainly there are those who consider the protective part played by the leucocytes to be quite small, relatively speaking or merely incidental. That they can and do ingest foreign particles and are subsequently to be found in the various tissues bearing their loads, is however, proved. It is only the interpretations laid on the facts that differ.

Many experiments have been made from a pathological standpoint to prove, if possible, the true part played by the various tissues and cells in diseases, which owe their existence to the presence of foreign matter or foreign organisms in the body. Naturally from the medical standpoint these experiments have been made on mammals of various kinds, the only other animal used being the ever useful frog. In contrast to this basis of work is the normal physiological condition existing under ordinary circumstances in animal life. All the experiments, of which the results are here given, were made under as purely normal conditions as possible, in every way anything that might produce abnormal results, being avoided. The animals used for these experiments were the two salamanders, *Necturus maculatus* or the Mud-puppy and *Cryptobranchus alleghaniensis*, the Hell-bender.

For several reasons these animals afford peculiar advantages for an investigation of this kind. Of great importance among these is the large size of the leucocytes and of the various tissue cells, and also the comparatively simple structure of the different organs. Another great advantage in the study of the living leucocytes lies in their activity in the ordinary temperature of a room, a fact, which affords an opportunity for the close observation of the process of ingestion. By mixing on a slide a small drop of fresh blood or lymph with a small quantity of lamp-black suspended in normal salt solution, the taking up or ingestion of the carbon by the leucocytes can be seen to take place while they pass through their amoeboid phases. In a few hours the cells become filled with carbon particles (Pl. IV), which are, however, contained exclusively in the cell body although appearances suggest their presence in the nuclei. These latter parts also exhibit amoeboid forms (Pl. IV). By watching the cells carefully the granules are seen to move across the nuclei and gradually leave it clear, proving beyond doubt that they are in the cell body.

In introducing the carbon into the living animals the following method was used. Into the abdominal cavity of the animals from $\frac{1}{4}$–1 c. c. of a mixture of lamp black, gum arabic and normal salt solution was injected. Here it should be said that in these animals this cavity forms practically a great lymph space, in which the carbon is ingested by the leucocytes, the latter then pass into the blood circulation and from that to the various organs and tissues. After periods varying from 4–10 days different animals were killed and the blood and tissues examined. In the case of Necturus, owing to the presence of external gills, the time of the appearance of the carbon-laden cells in the blood could be easily determined. By etherizing the animals and microscopically examining the circulation of the blood in the gill filaments once or twice a day the time of the appearance and also of the disappearance of ingested cells can be noted. The earliest appearance was on the 6th and the latest on the 9th days after injection. After 16 days a few scattered cells still remained. The results now given were chiefly obtained from a specimen of Cryptobranchus killed 10 days after injection.

In the microscopical examination of the tissues the first difficulty encountered lay in the presence of a large amount of natural pigment in the tissues. This is confusing both from the similarity in colour and from the necessary obscuring of structural parts. Caustic potash destroys melanin, but boiling is required and that of necessity injures the tissues. Ether, alcohol, acids and strong alkalies will also remove the colour, but the last two destroy the tissues and the first two decolorize so slowly as to be practically useless. By means of hydrogen dioxide the most successful results were obtained. The sections when cut and fastened to the slide were put in a vial of a 2% solution of the liquid. In from 6–48 hours, depending on the amount of pigment present, the color is reduced from black to a pale yellow without any attendant injury to the tissues. The process of decolourization is materially hastened by placing the vial containing the liquid and tissue in the strong sunlight and if desired all traces of the pigment can be removed. Practically it was found to be a great advantage to leave sufficient colour to mark the position of the pigment-bearing cells. By this method the black ingested leucocytes were easily distinguished wherever they occurred, and no chance for confusion remained.

Serial sections were made of the following parts: the spleen, kidney, ureters, liver, lung, stomach, muscle and skin. In all these parts ingested cells were present, but the positions and relations differed somewhat with the different organs. In the kidney (Pl. VI) carbon-laden leucocytes were in the blood capillaries, in the glomeruli, in the lymph spaces surrounding the capsules of the glomeruli, in the urinary tubules and in the nephrostomes. These latter parts are peculiar structures present in the amphibian kidney and are marks of a much more primitive form of that organ than exists in mammals. They consist of small ciliated funnels opening on the ventral surface of the kidney directly into the abdominal cavity. A small tube then unites these funnels with the urinary tubule arising from the glomeruli. The ingested leucocytes were in these funnels and by a series of sections they could be found to pass down the tube and into the urinary tubule. No doubt the number of leucocytes that pass from the blood circulation into the tubules is largely increased by additions from this source. No signs of ingested leucocytes in other than these places were found, or any trace of free carbon. Serial sections made of the ureters (Pl. VI) close to their openings into the cloaca showed masses of ingested cells. This indicated that a considerable number of such cells found their way out of the body in this way. Uningested cells were also found among those containing carbon. In the liver (Pl. V) ingested cells were found in the blood vessels alone. No extra-vascular carbon-laden leucocytes were present. In the stomach (Pl. VII) the carbon-laden cells were in the blood-vessels, in the epithelial tissue of the stomach and free on the inner surface, showing a gradual passage from the vessels to the epithelial surfaces. In the lungs (Pl. VI) practically the same time condition existed and also in the skin (Pl. VII). In the latter leucocytes could be traced from the blood-capillaries through the various layers and finally free on the outer surface of the skin. That these outside had not come from accidental external contact was proved by the fact that no red corpuscles were among these leucocytes and with the very rapid coagulation[1] that takes place in amphibian blood it would be impossible for the white cells to be completely isolated from the red. In various parts of the muscular tissue, either in the lymphatics or simply between the muscular fibres, ingested cells occurred rarely.

In all these parts there was absolutely no evidence for the presence of free carbon or carbon in any other cells than leucocytes. When, however, the spleen (Pl. V) was examined some peculiar and very interesting differences were found. The carbon was contained in leucocytes of a similar nature to those in previous cases, but in addition round the malpighian corpuscles there was what seemed at first sight to be a free deposit of carbon. But when carefully observed the carbon proved to be contained in cells that from their position were judged to be spleen-pulp cells. The distribution of the carbon in these cells differed exceedingly from that present in the leucocytes. Instead of being massed irregularly the carbon was evenly scattered through the cells, and, owing to the extended condition of the latter, covered a large area. When the fact of the presenee of carbon in these cells was established the question of the means of the transfer of the carbon from the leucocytes to the spleen cells at once arose. It was already proved that no free carbon entered the blood circulation. Consequently the spleen cells must have obtained their foreign material either directly or indirectly from the already ingested leucocytes in the blood. Two ways are open for this to take place. The leucocytes may in some manner discharge their load, which is afterwards taken up by the spleen cells, or the spleen cells may ingest the leucocytes and consequently the carbon. The latter seems to be the most plausible explanation. Moreover from the amount of carbon contained in the spleen cells the number of leucocytes destroyed in this manner must be considerable.

A brief summary of the results of the experiments is contained in the following statements:

1. No free carbon was present in any part examined.
2. All carbon was contained in leucocytes except in the spleen, where true splenic cells also contained it.
3. Ingested cells were both extra- and intravascular, except in the liver.
4. Ingested cells were free on mucous and epidermic surfaces; in the stomach, lungs and skin.
5. Ingested cells were in excretory organs with waste products, kidneys.

From the above results it is seen that the number of leucocytes in the body suffers a constant loss in three ways, by the wandering out of the cells on mucous and epidermic surfaces, by passing away with waste products and through ingestion by the splenic cells. The large numbers found in all three conditions show that the destruction of leucocytes through these ways is by no means insignificant. Moreover as no pathological conditions, so far as could be determined, were induced in the animals by the treatment, there is no reason to believe this loss to be other than a normal occurrence.

This method of removing the artificially introduced material by the leucocytes suggests at least the manner of the removal of any foreign matter that may enter the circulation during life. The leucocytes thus perform the duties of scavengers of the body in addition to their other important duties, even if by the very assumption of this office, they ultimately become waste material and as such pass away from the system.

One of the most interesting of the many problems that, even in these few experiments, have presented themselves, remains as yet unsolved. Owing to want of time the ultimate fate of the carbon contained in the spleen-pulp cells remains unascertained, nor can any suggestions be offered. Only after more prolonged experiments could it be determined whether the carbon disappeared from the cells or remained permanently in them. After the determination of this point if the first condition was found to obtain, the question as to the method of this removal would remain to be settled. In all the problems connected with the blood and circulation this perplexing organ seems to play an important part and when, setting aside function, differences of opinion exist as to structure, it can easily be seen that discussion on this part of the experiments involves doubtful and difficult problems. As is usual in any investigation many doubtful points have been raised that yet await settlement, leaving an interesting and fruitful field for further work.

NOTE.—The author wishes to express her indebtedness to the American Microscopical Society for the use of plates illustrating this article.

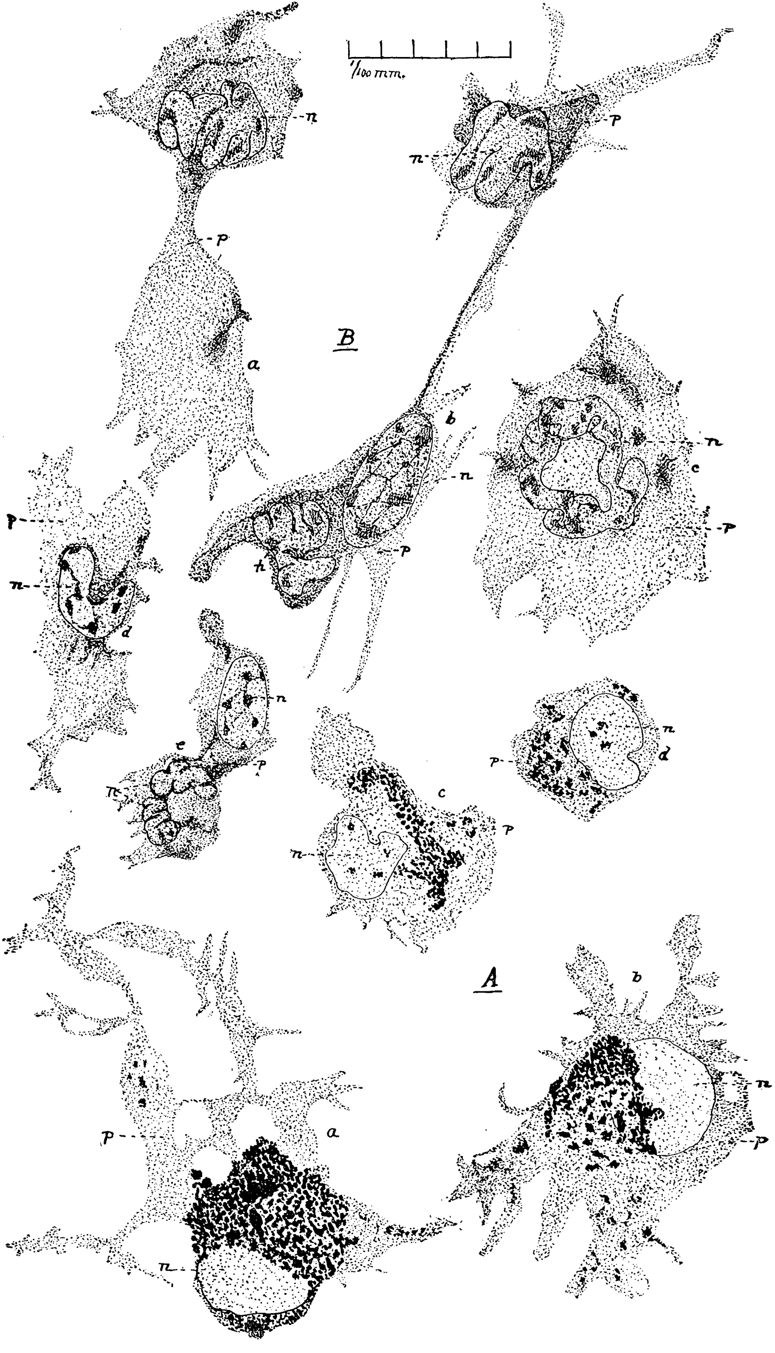
PLATE IV.

Leucocytes.

A. Group of carbon-laden leucocytes showing amoeboid phases.
  a. b. Leucocytes of Necturus.
    n. Nucleus.
    p. Cell-body.
  c. d. Leucocytes of Cryptobranchus.
    n. Nucleus.
    p. Cell-body.
  Drawn from dried preparations.

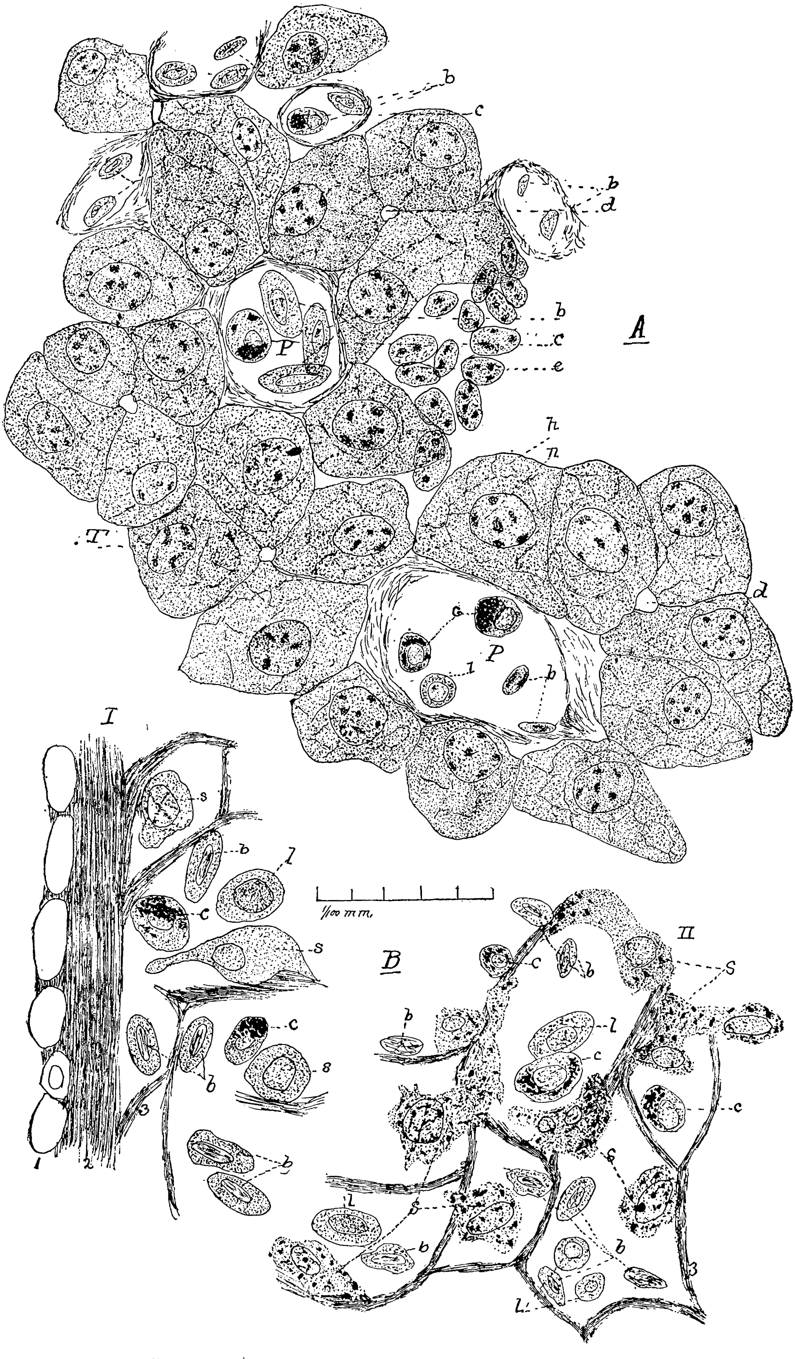
B. Group of Leucocytes, showing amoeboid cell-bodies and amoeboid nuclei.
  a. b. c. Leucocytes of Necturus.
    n. Nucleus.
    p. Cell-body.

PLATE IV.



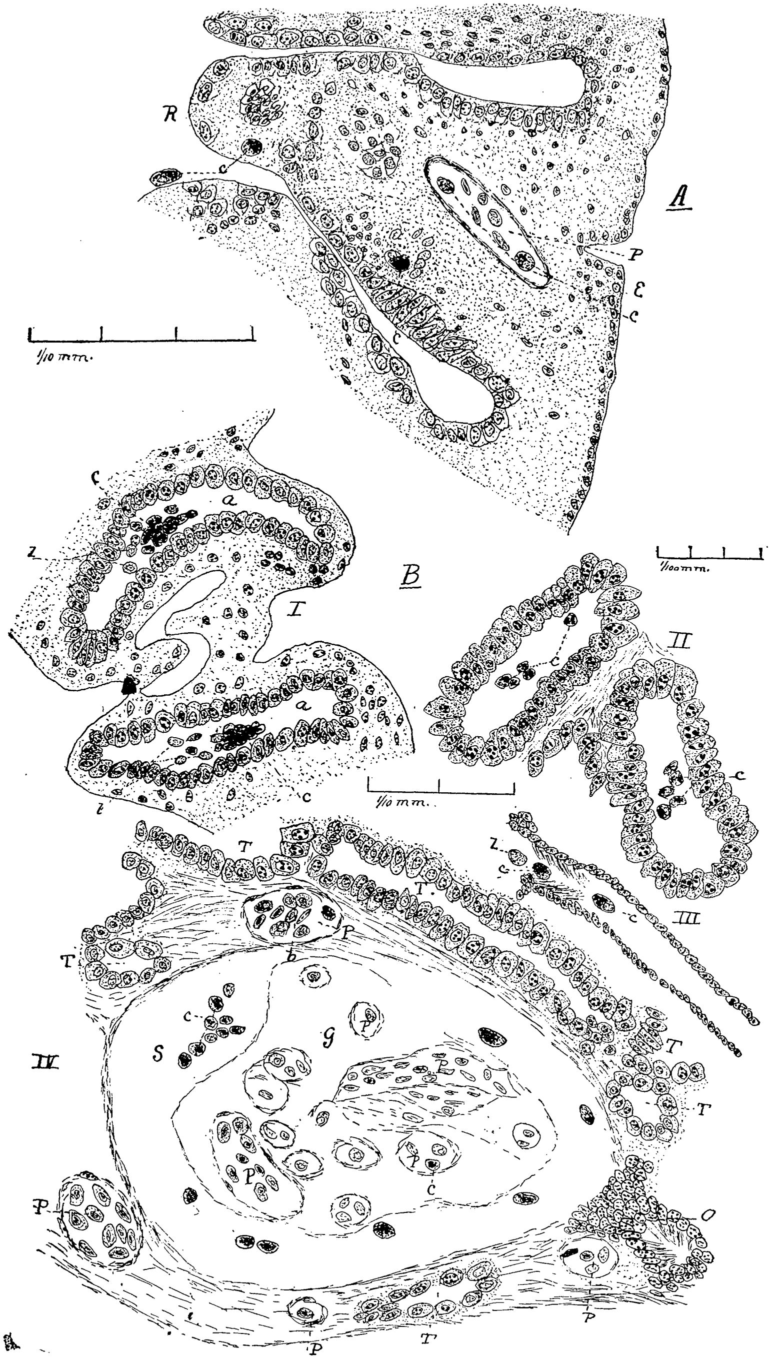
Leucocytes of Necturus and Cryptobranchus.

PLATE V.



Sections of Liver and Spleen of Cryptobranchus.

PLATE VI.



Sections of Lung and Kidney of Cryptobranchus.

b. Shows three nuclei, two in amoeboid movement and one resting.
d. e. Leucocytes of Cryptobranchus.
  n. Nucleus.
  p. Cell-body.
e. Has three nuclei, two amoeboid and one resting.

Drawn from stained preparations.

Plate V.

A. Surface section of liver of Cryptobranchus.
  p. Capillaries of blood-vessels.
  t. Liver-cells.
  b. Red corpuscles in the capillaries.
  d. Small intercellular capillaries of bileduct.
  e. Epithelium of the larger bile vessels.
  h. Hepatic cell-body.
  n. Nucleus of hepatic cells with nucleoli.

Note the absence of extravascular ingested leucocytes.

B. Vertical section of the spleen of Cryptobranchus.
  I. Part of the section near the surface.
    1. Peritoneum.
    2. Layer of fibrous tissue forming the capsule.
    3. Trabeculæ passing from capsule among splenic cells.
    c. Carbon-laden leucocytes.
    b. Red corpuscles.
    l. Leucocytes, non-ingested.
    s. Splenic pulp-cells.
  II. Ental part of the section.
    Lettering as above.

Note the ingested spleen pulp cells and the different distribution of the carbon particles in them from that found in the leucocytes; also the absence of ingested splenic cells in the superficial part of the spleen.

Plate VI.

A. Vertical section of lung of Cryptobranchus.
  E. Ectal surface.
  R. Ental or respiratory surface.

P. Blood capillary.
c. Carbon-laden leucocytes.

Note the presence of ingested leucocytes in extravascular tissue as well as on the ental surface of the lung.

B. Section of kidney of Cryptobranchus.
  I. Transection of the ureters and cloaca, showing masses of ingested cells.
    a. Ureters.
    c. Carbon-laden leucocytes.
    l. Non-ingested leucocytes.
  II. Transection of urinary tubules.
    c. Carbon-laden leucocytes.
  III. Nephrostomic funnel, showing ciliated mouth.
    c. Ingested leucocytes.
    l. Non-ingested leucocytes.
  IV. Vertical section of the kidney near the ventral surface.
    G. Glomerulus.
    P. Capillaries of blood-vessels.
    T. Urinary tubules.
    s. Lymph space around the glomerulus.
    o. Origin of a urinary tubules, with small ciliated epithelium.
    b. Red corpuscles.
    c. Carbon-laden leucocytes.

Note the presence of extravascular ingested cells.

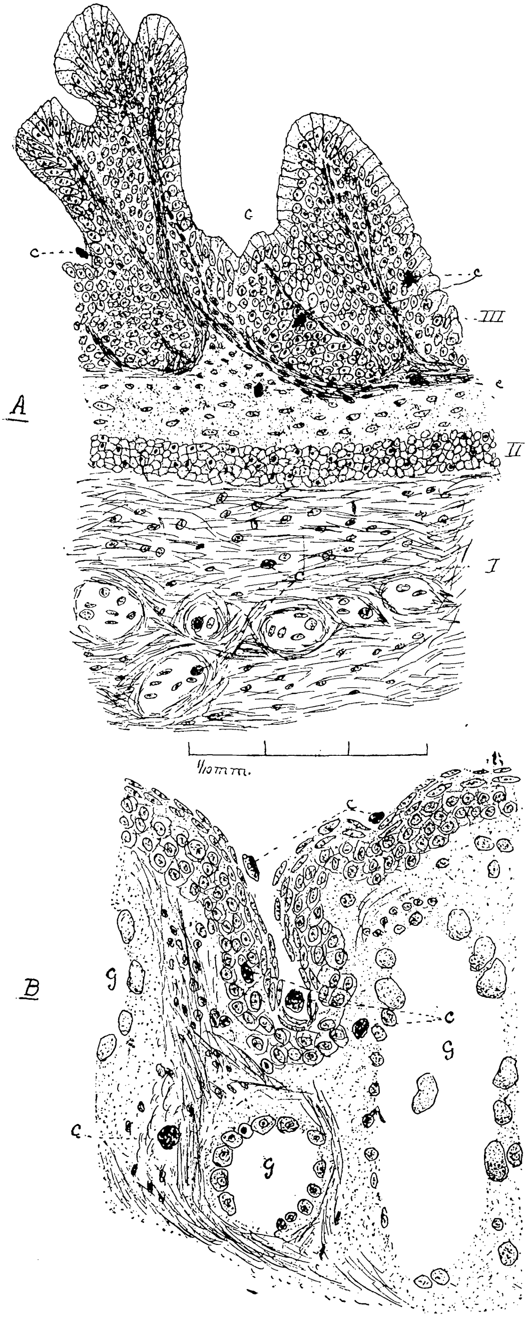
PLATE VII.

A. Vertical section of stomach of Cryptobranchus, near the pyloric part.
  I. Submucosa.
  II. Muscularis mucosæ.
  III. Mucosa.
    c. Carbon-laden leucocytes.

Note the presence of the extravascular ingested cells.

The figure is diagrammatic in so far that the locations of the ingested cells are taken from different sections and put into one figure.

PLATE VII.



Sections of Stomach and Skin of Cryptobranchus.

B. Vertical section of the skin of Cryptobranchus.
G. Large mucous glands.
c. Carbon-laden leucocytes.

The ingested-cells are wandering to the external surface from the blood-vessels.